# FURTHER STUDIES ON THE EFFECT OF VARIATIONS IN THE TEMPERATURE ON ANIMAL TISSUES.

ARTHUR W. GREELEY.

This paper contains an account of a series of experiments which are the outcome of others of a similar nature described in two earlier papers by the same author.[1] This previous work has called attention to the fact, noted by other observers, that the fluidity of the protoplasm of any of the Protozoa studied, varies directly with the temperature up to a certain critical point (28°–35° C.), above which the protoplasm suddenly goes into heat rigor, or coagulates. My own work has shown that as the temperature is lowered below the normal, a similar coagulation sets in which causes the cell to lose water.[2] This loss of water and coagulation is accompanied by a gradual cessation of the vital activities of the cell, and brings about certain very definite morphological changes that result in the formation of resting cells, which consist only of an undifferentiated mass of protoplasm. In the case of *Monas*, these changes were carried further by exposing the cells to a still greater reduction in the temperature, and the resting cells were finally broken up into many small spores, each of which reproduced the motile organism when returned to the normal temperature. As the temperature is raised above the normal, the protoplasm takes up water and all its vital activities are accelerated, until coagulation suddenly ensues at the critical point.

[1] Greeley, *American Journal of Physiology*, 1901, VI., p. 122. BIOLOGICAL BULLETIN, 1902, III., p. 165.

[2] This fact that lowering the temperature to 1° to 5° C. and raising the temperature above the critical point has the same effect upon protoplasm (*i. e.*, coagulation and loss of water), has received an interesting verification in the recent work of Fischer on Lepidoptera (*All. Zeit. für Entomologie*, October 15, 1901). In experiments on the artificial production of seasonal varieties of *Vanessa antiopa* by exposing the larvæ to different degrees of temperature, Fischer discovered that precisely the same variations in the adult forms are produced by lowering the temperature to 1° C. or raising it to 40° C., while modifications in the temperature within those limits gave strikingly different results.

In order to determine whether similar structural changes, as have already been described in the cases of *Stentor* and *Monas*, could be produced on other forms as well, the low temperature experiments have been continued on many other Protozoa, both Infusoria and Rhizopoda, and in all of them changes identical with those described above have been obtained. *Monas* is the only form in which it has been found possible to control the formation of spores, but in all the others resting cells were formed at the low temperature, which reverted to the motile condition when restored to the normal temperature.

## I. The Reversal of Vital Phenomena by a Reduction of the Temperature.

The results of these low-temperature experiments on the Protozoa suggested an interesting comparison to the experiment of Loeb's,[1] in which the tentacles and polyps of a Campanularian Hydroid were reduced to the undifferentiated protoplasm of the stolon by bringing them in contact with some foreign substance. It appeared that for the Protozoa a lowering of the temperature as well as a contact stimulus brings about just such a reversal of the vital phenomena until the undifferentiated resting cell is formed, while a small increase in temperature accelerates the metabolic processes. To see if a lowering of the temperature brought about similar changes in the more complex multicellular animals a series of experiments was begun on the fresh-water Hydra.

It was at once observed that whenever a Hydra is exposed to a temperature of 4° to 6° C., the tentacles gradually become shorter and thicker, and are finally completely absorbed into the body. As the absorption goes on, the ectoderm and entoderm cells of the tentacles lose their individuality and form an undifferentiated mass of protoplasm, which is slowly taken into the body of the Hydra (see Fig. 4). The tentacleless body of the Hydra becomes slowly resolved into a dense spherical mass of coagulated protoplasm, in which no distinction between the individual cells can be made out, and remains in this condition as long as it is kept at a low temperature (see Fig. 3), but quickly forms tentacles and a double layer of cells again when it is re-

[1] Loeb: *American Journal of Physiology*, 1900. *IV.*, p. 178.

turned to the temperature of the room. Thus a lowering of the temperature seems to produce essentially the same effect on Hydra as the contact stimulus on the Campanularian Hydroid in Loeb's experiment. Likewise the structural changes appear to be identical with those produced by the low temperature upon the Protozoa.

Hydra react to variations in the temperature in another way which is interesting when compared to the reactions of Protozoa

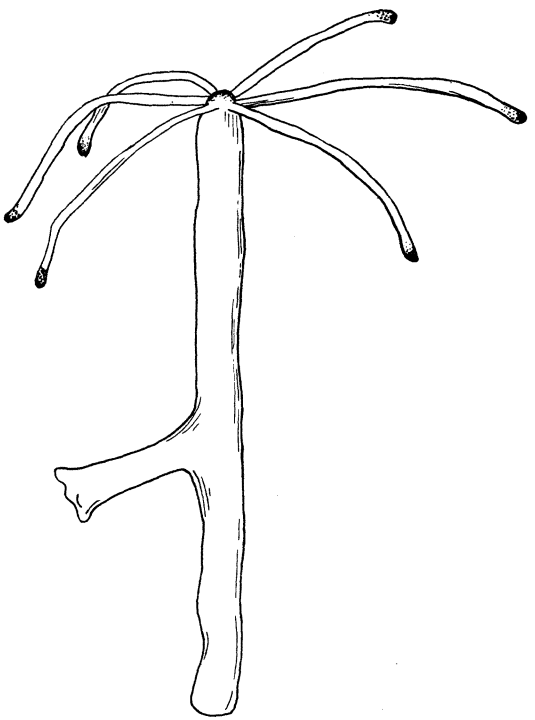

FIG. 1. A budding Hydra after an exposure of twenty-four hours to a tempera ture of 6°C. The body is slightly shortened and thickened, and the absorption of the ectoderm and entoderm cells has begun in the tips of the tentacles.

under the same conditions. It has been a fact of common observation that the rate of cell division varies directly with the temperature for all temperatures below the critical point. In my experiments on *Stentor* I showed that a lowering of the temperature not only inhibits cell division but brings about the reverse process. If a *Stentor* in the process of division be placed at a

temperature of 4°C. a fusion of the partially divided halves takes place. Among Hydra the formation of buds, which finally become distinct individuals, may be considered analogous to the process of cell division among Protozoa. It was found that if a Hydra in the earlier stages of the process of budding be placed

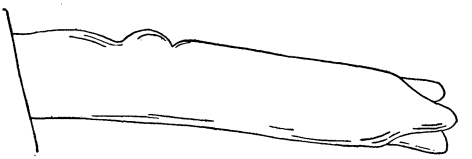

FIG. 2. The same Hydra as in Fig. 1, after an exposure of six days to a temperature of 6°C. The absorption of the tentacles and bud is nearly complete.

at a temperature of 4°C., not only does the growth of the bud stop instantly, but an absorption of the bud into the body of the parent commences, and continues until all traces of the bud have disappeared. (See Figs. 1 and 2.) In order to demonstrate this absorption of the bud, great care is needed in lowering the

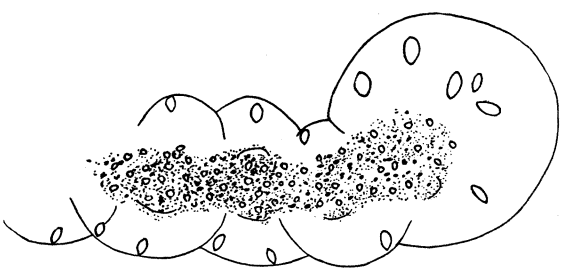

FIG. 4.

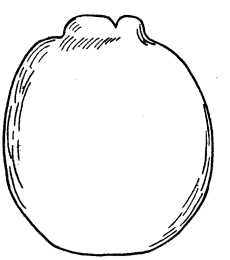

FIG. 3.

FIG. 3. The final resting stage of Hydra, formed after an exposure of four to seven days to a temperature of 6°C. The body consists of an undifferentiated mass of protoplasm.

FIG. 4. The tip of a tentacle of a Hydra that has been exposed to a temperature of 6°C. for twenty-four hours, showing the dissolution of the octoderm and endoderm cells.

temperature. If the temperature is quickly reduced to 1°C. the Hydra go to pieces, but if the temperature be maintained at from 4° to 6°C., and is not suddenly varied in either direction, the process of absorption can be easily seen. Six or seven days are required for the complete disappearance of the bud. These two experiments seem to show that among the Cœlenterates as well

as the Protozoa, a lowering of the temperature brings about a reversal of the vital phenomena and the formation of simple resting stages.

## II. The Effect of Variations in the Temperature Upon Developmental Processes.

It has been frequently observed that eggs, spores, cysts, or other resting stages of the motile organism which are formed to tide over some unfavorable conditions in the life-history of the animal, will not develop into the motile form unless they are exposed to the very conditions that brought about their formation, and normally intervene before development commences. Thus Braem[1] found that the statoblasts of Bryozoa, and the winter eggs of *Apus* would develop only after they had been exposed to a certain degree of low temperature. In this case the physical change produced in the protoplasm of the egg or statoblast by the low temperature seems to be necessary before the developmental processes can originate.

Dr. Loeb suggested to me the possibility that the same thing might be true for the development and metamorphosis of the chrysalids of a common moth, *Cecropia*, that are formed in the Autumn, but do not complete the metamorphosis until the following Spring. To test this hypothesis and see if other means besides that of low temperature would suffice to start development, the following series of experiments was performed:

On October 15, 1901, before they had been exposed to any frosts, a large number of cocoons were brought into the laboratory. Many of these chrysalids were found to be parasitized by an ichneumon fly, and only a small number were available for the experiments. The cocoons were kept in the laboratory at a temperature of 20° C. until November 27. They were then divided into two lots. One lot was kept constantly at a temperature of 20° C., as a control series, and the other was placed outdoors for six days, at a time when the temperature fell below 0° C. each night. At the end of the six days, these cocoons were brought back into the laboratory, and kept with the others at a temperature of 20° C. On January 27, the chrysalids that had

[1] Braem, *Jahrber. Schles. Ges. f. nat. Cult.* (Zool. Bot. Sec.), 1895, p. 2.

been exposed to the low temperature began to produce moths, and all of them had completed the metamorphosis by February 3. None of the chrysalids that had been kept at a temperature of 20° C. showed any signs of development. Several of the cocoons were opened, but the chrysalids were in the same condition, as far as could be seen, as when they were collected.

This result indicated that a lowering of the temperature at least accelerates the metamorphosis of the chrysalids. To determine whether the effect of the low temperature on the larva consisted in an extraction of water from the protoplasms, as was the case in the low temperature experiments on the Protozoa, the experiment was now varied as follows: The cocoons, that had been kept constantly at a temperature of 20° C., were now, on February 3, divided into three lots. One lot was retained at the room temperature, 20°C., another lot was exposed outdoors to a temperature of about — 10° C. for seven days, and the third lot of four cocoons was placed in a desiccator over sulphuric acid for two days. These four cocoons, while in the desiccator, lost water as is shown by the following record of weights:

| | Weight when Placed in Desiccator, Feb. 3. | Weight when Removed from Desiccator, Feb. 5. |
|---|---|---|
| 1. | 17.0555 g. | 17.031 g. |
| 2. | 15.184 g. | 15.173 g. |
| 3. | 16.630 g. | 16.603 g. |
| 4. | 15.115 g. | 15.095 g. |

These four cocoons produced moths on March 4, 10, 13 and 14. On March 24, moths emerged from the cocoons of the second lot that had been exposed to the low temperature, but on March 26 the cocoons of the control series that had been kept continuously at the room temperature produced moths also, showing that this last exposure to a low temperature was too late to have any effect. The desiccation hastened the development by about two weeks. We see from this experiment that the original exposure to a low temperature in November, soon after the cocoons were first brought into the laboratory, hastened the development by two months, and that the desiccation within two months before all the cocoons produced moths sufficed to accelerate the development materially. These experiments are far from satisfactory because of lack of material, but they furnish

testimony to the conclusion already reached by Braem and others, that in resting stages of this sort, development can commence only after some physical change has occurred in the protoplasm through the action of a low temperature or other changes in the external conditions. These experiments further seem to show that the changes produced in the protoplasm by lowering the temperature are identical with those produced by an extraction of water, as has already been indicated in the experiments on Protozoa.

It is interesting to note that the same forms of stimuli (*i. e.*, a lowering of the temperature and an extraction of water), which hasten the development of the moth, also produce artificial parthenogenesis of the starfish egg. This fact lends weight to the idea, expressed by Loeb, that artificial parthenogenesis consists merely in the acceleration of developmental processes already present in the egg.

## III. Effect of Temperature on the Absorption of Water by Muscle.

If the conclusion drawn from these earlier experiments, that a reduction of the temperature produces changes in the protoplasm that cause it to lose water is true, then variations in the temperature ought to have a decided effect on the absorption of water by muscle or other animal tissue. The experiments of Loeb[1] and Webster[2] on the gastrocnemius of the frog have demonstrated that this muscle always behaves in a very constant way, as far as can be determined by its change in weight, toward each salt solution in which it is immersed. In some salt solutions the muscle always absorbs a definite amount of water at the normal temperature, in others of the same osmotic pressure it always loses a definite amount. The only variation in this behavior of the muscle toward salt solutions occurs with the change of seasons, the muscle of winter frogs differing widely from summer frogs in this respect. This fact had been the only indication that temperature in any way affected the absorption of water by the frog's muscle, and the influence of the temperature

[1] Loeb, *Archiv. f. d. ges. Physiol.*, 1899, LXXV., p. 303.

[2] Webster, Univ. of Chicago Decennial Publications, 1902, X., p. 105.

alone was not clear in this case. In order to ascertain the influence of temperature upon this process and to obtain, if possible, some quantitative estimate of its action, I started a series of experiments to test the absorption of water by frog's muscle in the same solution at different temperatures.

All the salt solutions were used at dilutions isotonic with *m*/8 NaCl which is supposed to represent as nearly as possible the average osmotic pressure of the muscle. When tested at the normal temperature (20°C.), the solutions of all the salts experimented with, fall into one of three classes: first, those solutions which cause the muscle to absorb water as is shown by its increase in weight, for example, the univalent salts, KCl and $NH_4Cl$, and salts with a bivalent anion and two univalent kations as $Na_2SO_4$; second, those solutions which cause the muscle to lose water as shown by its decrease in weight, for example, salts with a bivalent kation and two univalent anions like $CaCl_2$ or or $SrCl_2$; third, those solutions which leave the water content of the muscle unaltered. LiCl is the best example of this third class. *m*/8 NaCl usually falls in this group, although in my experiments, I found that *m*/8 NaCl caused a slight increase in weight, and that *m*/6 NaCl was more nearly isotonic with the muscle.

The method used in the experiments was the same one that has been elaborated so successfully by Webster.[1] A large amount of each solution was made up isotonic with *m*/8 NaCl, and was then divided among dishes which were kept constantly at the following temperatures: 1°C., 20°C., 25°C., 27°C., 29°C., 31°C., 36°C., 38°C., 45°C. and 55°C. The gastrocnemius muscle of the frog was used in the experiment. The muscles were carefully weighed and then distributed among the dishes containing the solution to be tested at the temperatures named above. The muscles were weighed after remaining in the solutions for three hours, and again after twenty-four hours, and the gain or loss in the water content calculated in percentages of the original weight of the muscle.

The results of the experiments are given in Table I., in which are given curves showing the effect of temperature on the absorp-

[1] Webster, *loc. cit.*

tion or extraction of water after the twenty-four-hour exposure to each of the solutions. The curves for the three-hour exposure to the solutions are not given, because the twenty-four-hour

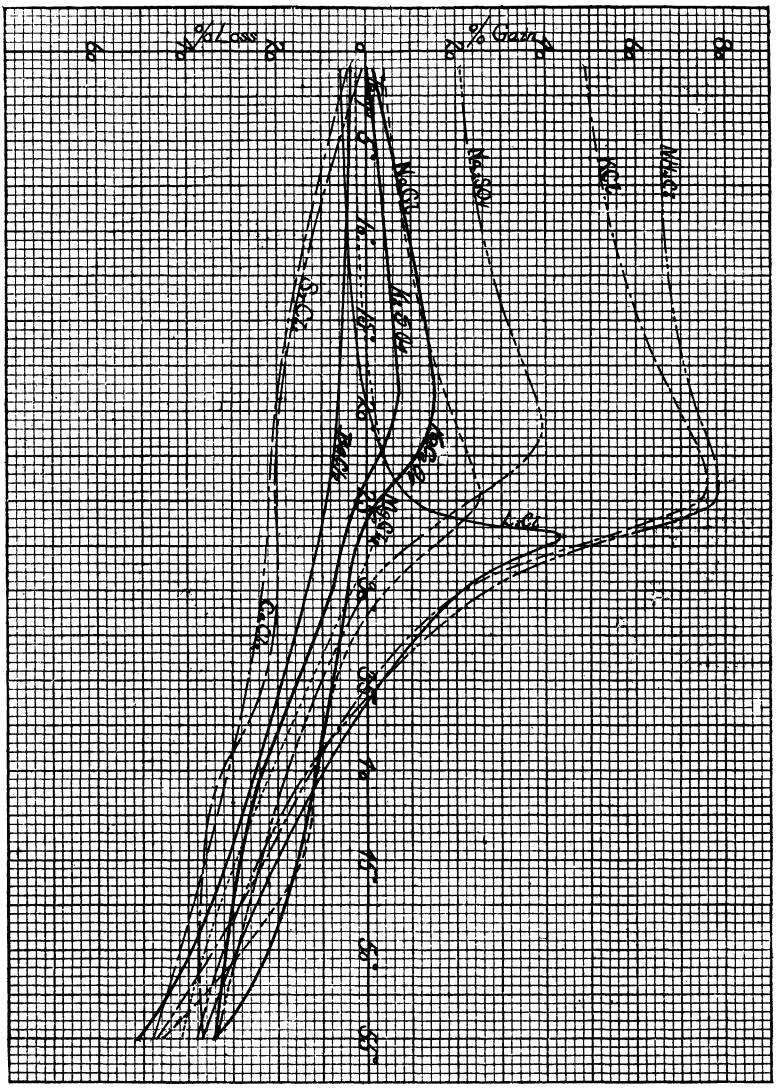


TABLE I. Curves showing the effect of temperature on the absorption of wat er by the gastroc-nemius muscle of the frog in various solutions isotonic with *m*/8 NaCl. The period of exposure to the solutions was twenty-four hours.

curves are entirely typical of the results obtained in both cases.

In considering the temperature effects we may classify them as regards their bearing on the absorption phenomena in the three classes of solutions mentioned above.

First, of those salts whose solutions, isotonic with *m*/8 NaCl, cause an absorption of water at the normal temperature (20°C.), the following were used: NaCl, KCl, $NH_4Cl$, $Na_2SO_4$, $K_2SO_4$ and $K_2C_2O_4$. In all of them, as is shown by the curves, the absorption of water varies directly with the temperature up to a certain critical point, in the neighborhood of 25°C., at which a sudden loss of water commences which increases rapidly with a further elevation of temperature. The form of the curve is the same for all the solutions, regardless of whether the initial absorption is great or small, and at about 50°C. the loss of water becomes practically the same in all the solutions. The form of these curves is strikingly like that showing the direct effect of temperature upon the amount of water in protoplasm, independently of the specific action of any salt solution, as is shown by the curve for *m*/8 NaCl which approximates as nearly as possible the fluid which normally bathes the muscle during life, as far as its chemical composition is concerned. For these reasons it seems probable that the effect of temperature upon the absorption of water in these solutions is due to the physical changes induced by the variations in the temperature in the protoplasm of the muscle. The rise in temperature may also accelerate the specific chemical action of the solution upon the muscle proteids, but in any event this only increases the result produced by the temperature alone. The amount of water in the protoplasm of a Protozoan varies directly with the temperature up to the critical point which marks the beginning of heat rigor, and it is interesting to find that the same thing occurs in muscle when immersed in solutions which are isotonic with its own substance. Above the critical point the heat rigor causes the same loss of water in all the solutions regardless of their chemical composition.

The same temperature effects are still better shown in curves of the absorption in solutions of the second class, *i. e.*, those which cause neither a gain or loss of water in the muscle at the normal temperature. Of the solutions of these salts, three were

used: LiCl, $MgCl_2$ and $m/6$ NaCl. 'Usually $MgCl_2$ has been described to act like $CaCl_2$, $BaCl_2$ and $SrCl_2$, in whose class it would naturally fall, in causing a loss of water. But although I tested its action many times, in all my experiments it had practically no effect on the weight of the muscle at the room temperature. It will be seen by examining the curves for LiCl and $MgCl_2$, that at a temperature of 1° C., the muscle loses a small amount of water. This loss of water decreases steadily as the temperature is raised until just above 20° C., an absorption of water commences, which increases until the critical point is reached. Above this point the muscles lose water very rapidly just as in the other solutions. Thus in these solutions, which appear to have no effect on the muscle at the normal temperature, there is a loss of water at low temperatures, a gain at temperatures between the normal and the critical point, and a very rapid loss above the critical point, which is exactly the effect that changes in temperature have been shown to have on the protoplasm of the cells of Protozoa, when only the physical condition of the protoplasm is modified by the variations in the temperature. $m/8$ NaCl also should have no effect on the amount of water in muscle at the room temperature, but in many cases, especially with the muscles of winter frogs, this solution appears to be hypotonic to the muscle substance. As Webster has shown, the osmotic pressure of the muscle varies with the season of the year, being higher during the winter, which is the condition we should expect from the observed effect of low temperatures on protoplasm. In my experiments (with winter frogs), $m/8$ NaCl invariably caused a slight gain in weight, but $m/6$ NaCl was found to be isotonic with the muscle, and the curve for this solution corresponds exactly with that for LiCl. Thus in all these solutions which appear to have no chemical effect on the muscle, as far as can be determined by the changes in weight, the amount of water in the muscle varies directly with the temperature up to the critical point, and inversely with the temperature above that point, and it is reasonable to suppose that the same thing occurs in the muscle in its normal surroundings within the body.

The curves for $CaCl_2$, $BaCl_2$ and $SrCl_2$ are very different from

those of the first two classes of salts. Solutions of these salts, isotonic with *m*/8 NaCl, cause a loss in weight of about 20 per cent. at a temperature of 20° C. It will be seen from the curves that in each of these solutions the loss of water is very slight at a temperature of 1° C., and that the decrease in weight increases steadily as the temperature is raised. But at a temperature of 50° the physical changes in the protoplasm overbalance the specific action of any solution and the muscles lose practically the same amount of water in all solutions. It appears that in the case of these solutions we are dealing with specific ion effects, as Loeb[1] has already suggested, and that the curve may be interpreted as follows: The speed of any chemical combination varies directly with the temperature. At a temperature of 1°C. the reaction between Ca, Ba or Sr and the muscle proteids is so greatly slowed that the solution has no effect on the muscle, and the small loss of water is due entirely to the physical changes in the muscle produced by the low temperature, as in LiCl, *m*/6 NaCl, and the other solutions which have no specific action on the muscle substance. As the temperature is increased, the reaction between the muscle proteids and the ions is accelerated, and this chemical action of the Ca, Ba or Sr ion overcomes the effect of the physical changes produced by the temperature, and the loss of water steadily increases because these ion proteid compounds, like Ca-soaps, hold very little water. It is worthy of notice, however, that at about 25° C. there is a break in the continuity of the curves, corresponding with the rapid absorption of water in the other solutions, which indicates a change in the physical condition of the protoplasm that neutralizes temporarily the specific ion effects.

In distilled water the amount of absorption by the muscle is decreased by lowering the temperature, as is shown by the following result for a one-hour exposure to distilled water: Percentage of absorption

| | |
|---|---|
| at 2° C. | 40.7 |
| at 30° C. | 53.1 |

[1] Loeb, *loc. cit.*

## Summary.

1. In Hydra as well as Protozoa, a lowering of the temperature brings about certain definite structural changes that result in the formation of an undifferentiated resting stage.

2. The inhibition of cell division and reversal of vital phenomena by a reduction of the temperature is shown in Hydra by the fact that at a temperature of 6° C., the growth of a new bud ceases, and the partially formed bud is gradually absorbed into the body of the parent animal.

3. A lowering of the temperature and an extraction of water both bring about the same physical changes in the protoplasm which serve to accelerate the development and metamorphosis of the chrysalids of *Cecropia*.

4. The absorption of water by the gastrocnemius muscle of the frog in those salt solutions which, when used at dilutions isotonic with its own substance, either have no chemical effect on the muscle at the room temperature, or cause an increase in weight, varies directly with the temperature, until the critical point is reached at which the muscle proteids begin to coagulate. In solutions of the same osmotic pressure, which cause the muscle to lose water at the room temperature, this loss of water varies directly with the temperature. Above the critical point of temperature the muscles lose practically the same amount of water in all solutions, regardless of their initial effect on the muscle.

Zoological Laboratory,
Washington University, March 18, 1903.